Reduced Frontal Glutamate + Glutamine and N-Acetylaspartate Levels in Patients With Chronic Schizophrenia but not in Those at Clinical High Risk for Psychosis or With First-Episode Schizophrenia

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Changes in brain pathology as schizophrenia progresses have been repeatedly suggested by previous studies. Meta-analyses of previous proton magnetic resonance spectroscopy (1H MRS) studies at each clinical stage of schizophrenia indicate that the abnormalities of N-acetylaspartate (NAA) and glutamatergic metabolites change progressively. However, to our knowledge, no single study has addressed the possible differences in 1H MRS abnormalities in subjects at 3 different stages of disease, including those at ultrahigh risk for psychosis (UHR), with first-episode schizophrenia (FES), and with chronic schizophrenia (ChSz). In the current study, 24 patients with UHR, 19 FES, 25 ChSz, and their demographically matched 3 independent control groups (n = 26/19/28 for the UHR, FES, and ChSz control groups, respectively) underwent 1H MRS in a 3-Tesla scanner to examine metabolites in medial prefrontal cortex. The analysis revealed significant decreases in the medial prefrontal NAA and glutamate + glutamine (Glx) levels, specifically in the ChSz group as indexed by a significant interaction between stage (UHR/FES/ChSz) and clinical status (patients/controls) (P = .008). Furthermore, the specificity of NAA and Glx reductions compared with the other metabolites in the patients with ChSz was also supported by a significant interaction between the clinical status and types of metabolites that only occurred at the ChSz stage (P = .001 for NAA, P = .004 for Glx). The present study demonstrates significant differences in 1H MRS abnormalities at different stages of schizophrenia, which potentially correspond to changes in glutamatergic neurotransmission, plasticity, and/or excitotoxicity and regional neuronal integrity with relevance for the progression of schizophrenia.

Key words: anterior cingulate cortex/at-risk mental state/biomarkers/frontal lobe/magnetic resonance imaging/neurochemical abnormality

Introduction

Identifying reliable biomarkers for the emergence and progression of schizophrenia is a fundamental priority for the development of efficient ways of detecting and protecting against the transition to psychosis and further progression.1,4 Although prefrontal or temporal gray matter (GM) volume reductions are promising candidate for the biomarker,5-10 neurochemical biomarkers have not been well established.

Proton magnetic resonance spectroscopy (1H MRS) allows for in vivo measurements of certain brain chemicals. These include N-acetylaspartate (NAA), an amino acid hypothesized to be a marker of neuronal integrity,11-14 and glutamate + glutamine (Glx), metabolites presumed to be involved in excitatory neurotransmission, plasticity, and excitotoxicity.14-19 Although 1H MRS does not selectively measure synaptic glutamate, brain glutamate abnormalities may be a major neurochemical contributor to schizophrenia.20-22 Previous studies have consistently reported lower-than-normal NAA and significant deviations in Glx levels in various brain regions, including the medial prefrontal cortex (mPFC), in patients with schizophrenia.12,23-26

A limited number of previous studies have compared 1H MRS metabolites across different stages of schizophrenia (ie, at ultrahigh risk for psychosis [UHR]).27 First-episode schizophrenia [FES], and chronic...
schizophrenia [ChSz]). One cross-sectional study examined 2 clinical groups of antipsychotic naive UHR and first-episode psychosis patients and reported increased glutamate levels in the dorsal caudate in both groups compared with controls.28 Bustillo and coworkers29 longitudinally studied NAA, including in frontal areas for up to 2 years, in patients with schizophrenia with less than 3 weeks of lifetime antipsychotic exposure. They reported lower-than-normal global NAA levels in the patients before treatment and no changes during follow-ups. Using a similar longitudinal design, a previous study reported higher-than-normal glutamine levels in the anterior cingulate and thalamus of never-treated patients with FES and later reductions of thalamic glutamine levels at a 30-month reexamination.30 Although these well-designed previous studies suggest how and when 1H MRS metabolite abnormalities emerge or progress, it would be difficult to implement long-term follow-up examinations across 3 different stages.

A recent meta-analysis25 reported NAA reductions in the frontotemporal cortices and thalamus of patients with schizophrenia and suggested that critical changes in the frontotemporal NAA abnormalities may occur in the transition from the premorbid stage to FES. A meta-analysis of Glx showed significantly decreased medial frontal glutamate and increased glutamine with not significantly lower Glx in patients with schizophrenia as compared with healthy individuals. It also reported that both Glx levels decreased faster-than-normal as the patients aged.15 These meta-analyses suggested that abnormalities in NAA, glutamate, and glutamine levels depend on the stage of the disease. However, to our knowledge, no previous study using a homogeneous study protocol has compared these metabolites in subjects with UHR, FES, and ChSz.

The current study was designed to examine whether the 1H MRS abnormalities in patients with schizophrenia depend on the stage of the disease. Based on the previous literature, it was reasonably expected that reductions in medial prefrontal NAA and Glx levels would be gradually prominent with the illness progression and would be most marked in patients at the chronic phase and relatively less evident in those at the FES and UHR. To test this hypothesis, our study examined clinical populations at the UHR, FES, and ChSz stages and 3 independent healthy control groups demographically matched to each of the 3 different stages. Because recent studies have pointed out methodological difficulties in independently quantifying Glx with a short echo time (TE) at 3-Tesla scanners,31-33 we utilized the composite Glx as a relatively reliable measure of the Glx.

Methods

Subjects

One hundred and forty-one Japanese participated in this study. Of these, 68 subjects were in the clinical groups, consisting of 24 UHR, 19 FES, and 25 ChSz. These subjects were recruited from the Department of Neuropsychiatry, The University of Tokyo Hospital, Japan. The diagnoses of schizophrenia for the ChSz patients were determined (paranoid [n = 17], disorganized [n = 3], catatonic [n = 1], undifferentiated [n = 2], and residual [n = 2]) according to the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) Axis I Disorder (SCID-I) Clinical Version.34 The inclusion criteria for FES and UHR described in our previous studies33,35 were used. Briefly, the FES subjects were 15–40 years of age, had received antipsychotic medications for less than 16 cumulative weeks, and had exhibited their first experience of psychosis according to the Structured Interview for Prodromal Symptoms (SIPS).36 Their diagnoses were confirmed as schizophrenia (paranoid [n = 18] and undifferentiated [n = 1]) over more than 6 months of examinations. The inclusion criteria for UHR were subjects between the ages of 15–30 years who had received a diagnosis of UHR according to the SIPS (attenuated positive symptom syndrome [APS] [n = 20], brief intermittent psychotic symptom syndrome [BIPS] [n = 1], genetic risk and deterioration syndrome without schizotypal personality disorder [n = 2], and comorbidity of APS and BIPS [n = 1]). Psychiatric symptoms for each patient were evaluated using the Positive and Negative Syndrome Scale (PANSS)37 within 7 days before and after their magnetic resonance imaging (MRI) scan. Fourteen UHR and one FES subject had not previously received antipsychotic medications, whereas the other patients were currently medicated with antipsychotics at the time of MR scans (only atypical antipsychotics [n = 38], only typical antipsychotics [n = 3], and both types of antipsychotics [n = 12]). The subjects with UHR included those with comorbid major depressive disorder (n = 2), anxiety disorder not otherwise specified (n = 3), and adjustment disorder (n = 1). Similarities in clinical manifestations and cognitive deficits have been pointed out between schizophrenia, UHR, and autism spectrum disorders (ASD).38,39 Additionally, because ASD subjects show aberrant prefrontal metabolite levels,40 all the participants were confirmed not to have diagnosis of ASD according to the DSM-IV based on clinical histories from all subjects and their family members. All clinical evaluations were performed by a psychiatrist (T.N., N.I., H.I., or Y.T.) fully trained to maintain reliability and consistency.

Seventy-three healthy control subjects participated, and they were assigned to 3 groups based on their demographic information with fully blind to the 1H MRS data as in the similar process to the previous studies.35,41 The first normal control (NC) group was matched to the UHR (NCUHR, n = 26), the second was to the FES (NCFES, n = 19), and the third to the ChSz (NCChSz, n = 28). The controls were screened for neuropsychiatric disorders through the SCID-I Non-patient Edition.34 Assessments of the subjects’ and their parents’ socioeconomic status
(SES) were conducted using the Hollingshead scale,\textsuperscript{42} handedness using the Edinburgh Inventory,\textsuperscript{43} and pre-morbid intelligence quotients (IQ) using the Japanese version of the National Adult Reading Test.\textsuperscript{44,45}

The exclusion criteria for all groups were a current or past neurological illness, a traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min, a history of electroconvulsive therapy, and previous substance abuse or dependence based on clinical histories. Additional exclusion criteria for the controls were a history of psychiatric disease in the subjects or of axis I disorders in their first-degree relatives. The ethical committee of The University of Tokyo Hospital approved this study (no. 397 and 2226). After a complete explanation of the study to the participants, written informed consent was obtained from every individual (table 1).

**MRI Acquisition**

The methods for MRI and MRS acquisition, processing, and quantification were the same as in our recent study.\textsuperscript{46} Briefly, MRI data were obtained using a 3-Tesla scanner (GE Signa HDxt). Axial T2-weighted images—TE = 82.32 ms, repetition time (TR) = 4400 ms, field of view (FOV) = 240 × 240 mm\textsuperscript{2}, matrix = 256 × 256, slice thickness = 2.5 mm, number of axial slices = 62—were acquired for positioning of the volume-of-interest (VOI). Three-dimensional fast spoiled gradient recalled acquisition with steady state (3D-FSPGR) images—TE = 1.94 ms, TR = 6.80 ms, FOV = 240 × 240 mm, matrix = 256 × 256, flip angle = 20°, slice thickness = 1.0 mm, number of axial slices = 176—were acquired for tissue segmentation of the VOI. A trained neuroradiologist (W.G., H.S., and H.T.) evaluated the MRI scans and found no gross abnormalities in any of the subjects.

**1H MRS Acquisition**

A stimulated echo acquisition mode (STEAM) imaging sequence—TR = 3000 ms, TE = 15 ms, mixing time = 13.7 ms, 128 water-suppressed, and 8 water-unsuppressed averages—was used to obtain the proton MR spectra. The location of the VOIs was based on our previous studies.\textsuperscript{46,47} Briefly, in the mid-sagittal T2 slice, a VOI (20 × 20 × 20 mm\textsuperscript{3}) was placed closest to the most anterior part of genu of the corpus callosum with the center of the posterior plane of the VOI. This VOI contained predominantly the GM of mPFC, including primarily the anterior cingulate and paracingulate gyrus bilaterally (figure 1).

**Spectrum Quantification**

All spectra were quantified with LCModel (ver.6.2-3A). The raw spectral data were read into LCMgui in which spectrum processing was performed automatically. Based on the comparison of the in vitro spectra from measurements analyzed with the LCModel basis set, the absolute levels for 17 metabolites were estimated from the in vivo spectra. Among these, the current study focused on NAA, Glx, creatine and phosphocreatine (Cre), myoinositol (mI), and glycerophosphocholine and phosphocholine (Cho). Representative spectra from patients and controls are shown in figure 1.

**Spectrum Quality**

Only metabolite spectra that showed Cramer-Rao lower bounds (CRLB) <20% were included in the analysis. Additionally, full width at half maximum (FWHM) less than 0.16 ppm and signal-to-noise ratio (SNR) ≥5 were required for inclusion. Based on these criteria, all metabolites in all the 141 subjects were included in the analysis as shown in table 2. The spectrum quality of the included subjects was good (see online supplementary tables 1 and 2).

**Tissue Segmentation**

As in our recent study,\textsuperscript{46} 3D-FSPGR images were used to calculate GM, white matter (WM), and cerebrospinal fluid (CSF) volumes. To obtain the tissue-composition-corrected metabolite intensities, each metabolite value was corrected for the CSF content of the VOI using the following formula as in a previous study:\textsuperscript{46} corrected metabolite level = uncorrected metabolite level/(1−C), where C is the fractional CSF content of the VOI.

**Statistical Method**

All statistical analyses were conducted using PASW Statistics 18 (SPSS Inc). GM, WM, and CSF volumes within the VOI and demographic variables, including age, height, body weight, subjects’ SES, parental SES, handedness, and IQ, were compared using MANOVA with these indices as the dependent variables and with main factors of clinical status (patients/controls) and stage (UHR and NC/ChSz and NC/FES). The sex ratios were compared using chi-square tests between the patient and the matched controls. The significance level was set at a \( P < .05 \) without correction for multiple comparisons to strictly assess the effects of potential confounds.

For the group comparisons of metabolite levels, we employed a repeated-measures ANCOVA using metabolite concentrations as the dependent variables with 2 between-subject factors (clinical status: patients/controls; stage: UHR and NC/ChSz and NC/FES), 1 within-subject factor (metabolites: NAA, Glx, Cre, Cho, and mI). The current analysis treated metabolites levels as a within-subject factor because metabolite levels were suggested to be interrelated in previous studies\textsuperscript{49–53} as well as in
Table 1. Clinical and Demographic Characteristics of the Subjects

<table>
<thead>
<tr>
<th></th>
<th>NC_{UHR} (n = 26)</th>
<th>UHR (n = 24)</th>
<th>NC_{FES} (n = 19)</th>
<th>FES (n = 19)</th>
<th>NC_{ChSz} (n = 28)</th>
<th>ChSz (n = 25)</th>
<th>Clinical Status ( \times ) Stage</th>
<th>( F(1,135) )</th>
<th>( P ) Value</th>
<th>( F(2,135) )</th>
<th>( P ) Value</th>
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<tbody>
<tr>
<td>Sex</td>
<td>13/13</td>
<td>12/12</td>
<td>14/5</td>
<td>14/5</td>
<td>17/11</td>
<td>15/10</td>
<td></td>
<td>0.003\textsuperscript{a}</td>
<td>.96\textsuperscript{a}</td>
<td></td>
<td></td>
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<tr>
<td>Age (range)</td>
<td>22.3 (16–26)</td>
<td>21.7 (16–29)</td>
<td>26.3 (21–29)</td>
<td>25.4 (17–37)</td>
<td>32.8 (27–41)</td>
<td>32.7 (27–50)</td>
<td></td>
<td>0.96</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SES\textsuperscript{b}</td>
<td>2.0 (2.0–4.0)</td>
<td>1.3 (2.9–5.0)</td>
<td>1.4 (1.4–1.5)</td>
<td>1.5 (1.7–1.7)</td>
<td>1.7 (1.7–1.7)</td>
<td>1.3 (1.7–1.7)</td>
<td></td>
<td>.47</td>
<td></td>
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<td>Parental SES</td>
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<td>0.8 (0.8–1.0)</td>
<td>1.8 (1.8–1.8)</td>
<td>0.6 (2.0–2.0)</td>
<td>0.6 (2.0–2.0)</td>
<td>0.6 (2.0–2.0)</td>
<td></td>
<td>.03</td>
<td></td>
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<tr>
<td>Height</td>
<td>166.0 (89–165.5)</td>
<td>165.5 (7.7)</td>
<td>173.1 (9.8)</td>
<td>165.7 (8.6)</td>
<td>167.5 (7.9)</td>
<td>164.9 (8.7)</td>
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<td>1.78</td>
<td>.19</td>
<td></td>
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<tr>
<td>Weight</td>
<td>58.3 (11.2–57.4)</td>
<td>57.4 (8.7)</td>
<td>65.4 (9.3–13.3)</td>
<td>60.7 (9.9)</td>
<td>60.7 (9.9–11.5)</td>
<td>66.6 (14.5)</td>
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<td>2.83</td>
<td>.09</td>
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<td>Handedness\textsuperscript{d}</td>
<td>85.0 (40.4)</td>
<td>87.0 (18.8–93.9)</td>
<td>94.6 (11.5)</td>
<td>95.2 (8.6–93.7)</td>
<td>93.7 (13.8)</td>
<td>93.0 (91)</td>
<td></td>
<td>3.6 (0.6)</td>
<td></td>
<td>2.1 (0.13)</td>
<td></td>
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<tr>
<td>IQ (JART25)\textsuperscript{e}</td>
<td>106.8 (10.3)</td>
<td>108.4 (9.1–109.0)</td>
<td>103.2 (13.3)</td>
<td>108.5 (9.3–102.7)</td>
<td>11.3 (3.6)</td>
<td>2.1 (0.13)</td>
<td></td>
<td>0.009</td>
<td></td>
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<tr>
<td>PANSS</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Positive symptoms</td>
<td>13.6</td>
<td>2.9</td>
<td>16.0</td>
<td>4.5</td>
<td>16.2</td>
<td>6.0</td>
<td></td>
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<tr>
<td>Negative symptoms</td>
<td>18.1</td>
<td>6.3</td>
<td>18.8</td>
<td>4.8</td>
<td>21.6</td>
<td>7.1</td>
<td></td>
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<td>General</td>
<td>33.1</td>
<td>7.0</td>
<td>36.4</td>
<td>7.4</td>
<td>38.4</td>
<td>11.3</td>
<td></td>
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<td>GAF</td>
<td>48.0</td>
<td>11.8</td>
<td>38.4</td>
<td>8.4</td>
<td>38.3</td>
<td>14.3</td>
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<tr>
<td>Onset of illness (y)</td>
<td>25.1</td>
<td>6.0</td>
<td>25.4</td>
<td>7.7</td>
<td>25.4</td>
<td>7.7</td>
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<tr>
<td>Duration of untreated psychosis (wk)</td>
<td>15.7</td>
<td>20.9</td>
<td>45.4</td>
<td>77.2</td>
<td>92.8</td>
<td>59.2</td>
<td></td>
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<tr>
<td>Duration of illness (mo)</td>
<td>8.4</td>
<td>10.0</td>
<td>10.0</td>
<td>59.2</td>
<td>92.8</td>
<td>59.2</td>
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<tr>
<td>Antipsychotic dose\textsuperscript{f} (mg/d)</td>
<td>117.6</td>
<td>194.6</td>
<td>558.1</td>
<td>512.9</td>
<td>839.8</td>
<td>845.0</td>
<td></td>
<td></td>
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<tr>
<td>Antipsychotic type (atypical/typical/both/none)</td>
<td>9/0/1/14</td>
<td>16/1/1/1</td>
<td>13/2/10/0</td>
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</table>

Note: NC, normal control; UHR, ultrahigh risk for psychosis; FES, first-episode schizophrenia; ChSz, chronic schizophrenia; SES, socioeconomic status; IQ, intelligence quotients; PANSS, Positive and Negative Syndrome Scale; GAF, Global Assessment of Functioning.

\( \textsuperscript{a} \) Chi-square test for ChSz stage.

\( \textsuperscript{b} \) Assessed with the Hollingshead scale. Higher scores indicate lower educational and/or occupational status.

\( \textsuperscript{c} \) Post hoc test indicated that the patient group was significantly different from each control group (\( P < .05 \), independent 2-tailed \( t \) tests).

\( \textsuperscript{d} \) Determined with Edinburgh Inventory: scores more than 0 indicate right handedness. A score of 100 indicates strong right handedness.

\( \textsuperscript{e} \) Estimated from scores on the Japanese Adult Reading Test.

\( \textsuperscript{f} \) Based on chlorpromazine equivalents.
the present study (ie, most metabolites pairs of all of the 10 possible combinations of 5 metabolites showed significant correlations: 9 of the 10 pairs at $P < .05$ and 7 of 10 at $P < .005$ Bonferroni corrected for 10 pairs). Three covariates (age, parental SES, and GM volume) were also included in the analysis because significant differences between different clinical status or stages were found for age and parental SES. Because the CSF components had already been accounted for by calculating the corrected metabolite level (see “Tissue Segmentation” section), GM components were further treated as covariates to account for possible difference in metabolite levels between GM and WM. If a significant interaction between clinical status and any other factor was found, post hoc analyses were performed using repeated-measures ANCOVA (see “Results” section). If a significant interaction between metabolites and any other factor was found, further post hoc analyses using multivariate ANCOVA (MANCOVA) with the level of metabolites as a dependent variable and age, parental SES, IQ, and daily antipsychotic dose based on chlorpromazine equivalents, were also tested separately in each diagnostic group using Spearman’s correlation coefficients. The threshold for statistical significance was set at $P < .001 = .05/54$ correlations using Bonferroni correction.

Furthermore, to test the potential interrelationship between the metabolites abnormalities, post hoc correlational analyses between NAA and Glx levels, which showed significant deviations from the normal in the ChSz (see “Results” section), were conducted in the patients with ChSz as well as UHR and FES. Considering the exploratory nature of the correlational analyses, the threshold for statistical significance was set at $P < .0017 = .05/30$ correlations using Bonferroni correction. Additionally, the correlations between NAA and Glx levels and potential confounding factors, including age, SES, parental SES, IQ, and daily antipsychotic dose based on chlorpromazine equivalents, were also tested separately in each diagnostic group using Spearman’s correlation coefficients. The threshold for statistical significance was set at $P < .001 = .05/6$ correlations using Bonferroni correction.

Results

Demographic and Clinical Measures
The MANOVA showed significant interaction of clinical status and stage on SES ($F[2,135] = 3.7$, $P = .03$), a main effect of clinical status in SES ($F[1,135] = 72.0$, $P < .001$), parental SES ($F[1,135] = 19.4$, $P < .001$), and
height ($t[1,135] = 5.7, P = 0.02$). The interaction between clinical status and stage and the main effect of clinical status were not significant for the other dependent variables ($P > .06$). Post hoc independent 2-tailed $t$ tests revealed significant differences as follows: compared with their matched controls, the subjects with ChSz ($t[51] = -7.49, P < .001$, FES ($t[36] = -4.94, P < .001$, and UHR ($t[48] = -2.72, P = .009$) had significantly lower SES. The subjects with ChSz and FES had significantly lower parental SES ($t[51] = -3.48, P = 0.001$ and $t[36] = -2.94, P = .006$, respectively) than their matched controls. The subjects with FES had significantly lower height ($t[36] = 2.45, P = 0.019$) than their matched controls (table 1).

### $^1$H MRS Measures

Repeated-measures ANCOVA showed a significant interaction between stage and clinical status ($F[2,132] = 4.98, P = 0.008$) with no significant main effect of stage or clinical status ($P > .12$) and no interaction between metabolite concentration and stage or clinical status. Post hoc repeated-measures ANCOVA for each stage with “clinical status” as a between-subject factor, “metabolites” as a within-subject factor, and the same covariates as the main analysis were conducted with the threshold for statistical significance set at $P < .017 = .05/3$ stages using Bonferroni correction. These post hoc analyses revealed a significant main effect of clinical status ($F[1,48] = 12.66, P = 0.001$) and a significant interaction between the metabolites and clinical status ($F[4,192] = 3.71, P = .006$) in the ChSz subjects, whereas there were no significant main effects of clinical status or significant interactions in the FES or UHR subjects. Post hoc MANCOVA for ChSz with clinical status as a main factor, metabolite concentrations as the dependent variables, and the same covariates as the main analysis revealed significant effects of clinical status for NAA + NAAG ($F[1,48] = 17.13, P < .001$) as well as Glx.

### Correlations With Metabolite Concentrations

Correlational analyses showed relationships between the Glx level and DOI ($r = 0.524, P = 0.021$) and DUP ($r = 0.638, P = 0.008$) in the FES, although not statistically significant after correction for multiple comparisons. Several correlations were found between the Glx level and parental SES in the NC$_{ChSZ}$ ($r = 0.422, P = 0.025$), the NAA level and SES ($r = 0.477, P = 0.039$), daily antipsychotic medication dose ($r = -0.491, P = 0.033$) in the patients with FES, and Glx level and age in the individuals at UHR ($r = -0.513, P = 0.010$). However, these correlations were not significant after correction for multiple comparisons.

In the patients with ChSz, the reduced NAA level was significantly correlated with the reduced Glx level ($r = 0.720, P < .001$) while not in the subjects with UHR or FES. The correlation was similar in pattern to those observed in the NC$_{ChSZ}$ ($r = 0.571, P = .001$) or for NC$_{UHR}$ ($r = 0.553, P = 0.003$).

### Discussion

The current study revealed significantly decreased medial prefrontal NAA and Glx levels specifically in the subjects at chronic stage of schizophrenia. The specificity of these
Table 2. Descriptive and Inferential Statistical Results of the Metabolite Concentrations in the Participants

<table>
<thead>
<tr>
<th>Metabolites (mmol/ml)</th>
<th>Clinical Status</th>
<th>Stage</th>
<th>Repeated-Measures ANCOVA: Main Analysis</th>
<th>Clinical Status × Stage</th>
<th>MANCOVA in Stage of ChSz (^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA</td>
<td>Controls</td>
<td>UHR</td>
<td>N  Mean  SD</td>
<td>F(1,132)  P</td>
<td>F(2,132)  P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26  8.00  1.22</td>
<td>2.42  .12</td>
<td>0.44  .64</td>
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Note: Abbreviations are explained in the first footnote to table 1. NAA, N-acetylaspartate; Glx, glutamate + glutamine; Cre, creatine and phosphocreatine; mI, myoinositol; Cho, glycerophosphocholine and phosphocholine. Bold values indicate statistical significance.

\(^{a}\)Post hoc MANCOVA for the stage of ChSz after the significant interaction of clinical status × metabolites in follow-up analyses in the stage of ChSz. Threshold for statistical significance was set at \(P < .01\) with Bonferroni correction.

\(^{b}\)Post hoc repeated-measures ANCOVA for each stage after the significant interaction of clinical status × stage in main analysis. Threshold for statistical significance was set at \(P < .017\) with Bonferroni correction.
reductions to the patients at chronic stage compared with
those at the UHR and FES stages was shown by a sig-
nificant interaction between diagnosis and illness stage.
The specificity of reduced NAA and Glx levels compared
with Cre, Cho, and mI levels was shown by a significant
interaction between diagnosis and metabolite types in the
patients with ChSz and their matched controls. Thus, the
present study demonstrates significant differences in
$^1$H MRS abnormalities between patients at different stages
of schizophrenia, which differentially occur with differ-
ent metabolites.

The chronic patients-specific decrements of medial
frontal NAA are generally in line with previous studies,
indicating robust reductions in medial frontal NAA in
chronic but not in early phase schizophrenia. Although
a meta-analysis of 97 $^1$H MRS studies$^{25}$ found a reduced
NAA in the frontal lobe, both in FES and ChSz, they
suggested that the criteria for FES were not explicitly
reported in any of the 19 studies included in their meta-
analysis. Furthermore, among the 19 studies, 7 of the 9
studies employed a medial frontal VOI reported no change
in NAA consistent with our study,$^{55,57–62}$ whereas the other
2 reported reduced NAA. One of these latter studies
recruited 13 children and adolescents with schizophrenia,
of whom only 3 subjects were first-episode patients.$^{63}$ The
other recruited 30 patients with and without Gilbert’s
syndrome, whose mean durations of illnesses were rela-
tively long with 1.3/1.7 years, respectively.$^{61}$ Thus, the cur-
rent and the latter 2 studies are different in the age, illness
stage, and mean durations of illness in the participants.
The current study suggests that there is no decrease in
the medial frontal NAA of FES, and this result does not
conflict with those of most of the previous studies.

We also observed significantly decreased medial
prefrontal Glx specifically in the patients with ChSz.
A meta-analysis of 28 $^1$H MRS studies in schizophrenia$^{15}$
found significantly decreased glutamate and increased
glutamine in medial frontal regions of patients with
schizophrenia compared with healthy controls. Our find-
ing of chronic patients-specific decreases in the medial
frontal Glx levels is in keeping with the result of this
meta-analysis that both Glx levels decreased at a faster-
than-normal rate with age in patients. Because the cur-
rent participants with FES were sufficiently treated with
antipsychotics, the preserved Glx level in the current
patients with FES is in line with a recent study which

![Fig. 2. Plots of the metabolite levels. Scatter plots showing the concentrations of (a) $N$-acetylaspartate (NAA), (b) glutamate +
glutamine (Glx), (c) creatine and phosphocreatine (Cre), (d) glycerophosphocholine and phosphocholine (Cho), and (e) myoinositol (mI)
in the subjects with the ultrahigh risk for psychosis (UHR), first-episode schizophrenia (FES), and chronic schizophrenia (ChSz) and
matched controls for each group. *Statistically significant at $P < .05$.](https://academic.oup.com/schizophreniabulletin/article-abstract/40/5/1128/1924003)
demonstrated significantly higher anterior cingulate glutamate/Cr or Glx/Cr in patients who were still symptom-atic than in those in remission.\textsuperscript{54}

Decreased NAA and Glx levels in ChSz may be associated with the progressive brain volume reductions in the mPFC that are associated with longer durations of schizophrenia.\textsuperscript{65-67} However, it is not possible to completely explain these metabolite decreases by volume reductions because of the use of tissue composition correction. Correlation between Glx and NAA in schizophrenia was previously reported in line with the N-methyl-d-aspartate receptor hypofunction model of schizophrenia\textsuperscript{14,56,68,69} and contributions from glutamate related dendritic toxicity. In accordance with the notion, the decreased NAA and Glx levels were significantly correlated in the patients with ChSz but not in the UHR or FES. Because Glx and NAA are thought to be inherently linked through a series of biochemical reactions, mainly the tricarboxylic acid and glutamate-glutamine cycles in neurons and glia,\textsuperscript{14,49,70} both of the altered Glx and NAA measures may commonly reflect dysfunction and/or loss of neuronal tissues. The specific loss of NAA and Glx, as compared with no change in Cho or mI, in the patients with ChSz is also consistent with previous reports, suggesting brain tissue loss occurs as a result of reductions of neuropil and potential rearrangements of cortical architecture, rather than by neuronal loss or degeneration\textsuperscript{15,71-76}.

Antipsychotic medications may have some effect on MRS measures.\textsuperscript{30,49,56,77-79} Most previous studies of unmedicated patients with schizophrenia have reported abnormally elevated glutamate or Glx levels in the mPFC\textsuperscript{30,51,57,58} with one study showing effects in the striatum.\textsuperscript{28} In addition, one previous study compared medial prefrontal Glx levels in medicated patients with those in unmedicated patients, and found elevated Glx levels only in the unmedicated patients.\textsuperscript{51} Even though no significant correlation between the daily antipsy- chotic dose and metabolite levels was found in the cur- rent patients with ChSz and no significant difference in any metabolites levels was found between the medicated (n = 10) and nonmedicated (n = 14) individuals at UHR (P > .2), the fact that all 3 patient groups were not all similarly medicated is a potential confound of the pres- ent study. Future study should adequately address the effects of antipsychotic medications on the Glx and NAA levels.

The current study extends the findings of previous meta-analyses by making direct comparisons in a single study. For the integration of findings across different studies, heterogeneity in magnetic field strength, acquisition mode, quantification method, and scanner type between studies should also be taken into account because these could have a significant impact on the variability of the metabolites quantified.\textsuperscript{5,77} Although meta-analyses cannot totally rule out these effects,\textsuperscript{15,25} our study confirmed ChSz-specific NAA and Glx abnormalities under uniform conditions in a single study.

There are several methodological considerations and limitations of our study. First, our MRS measurement was limited to the mPFC, even though other regions are likely involved in the pathology of schizophrenia. Although the single VOI model yields high S/N,\textsuperscript{90} future studies should examine the regional specificity of these findings. Second, although the present study examined patients at 3 different stages of schizophrenia, which would be difficult in a longitudinal study, the cross-sectional design supports descriptive rather than causal interpretations. Although 2 of the 24 individuals at UHR developed psychosis later in the mean of 12.4-month follow-up, in which the transition rate does not conflict with those in the previous studies,\textsuperscript{58} the small number of subjects with transition make any statisti-cal comparison difficult. Third, because MRS does not selectively measure synaptic glutamatergic metabo-lites, the results should be carefully interpreted regard-ing this matter. Fourth, the STEAM sequence with a TE = 15 ms left room for improvement, although the currently described low CRLB values indicate good Glx data quality. Future study should expand on the current findings using an optimized TE\textsuperscript{82,83} or improved handling of macromolecule signals.\textsuperscript{84,85} Fifth, although the main findings were based on the analysis controlling potential confounding effect of parental SES, a potential cohort effect (as distinct from a stage of illness effect) cannot be totally ruled out because of the lack of matching the sub-jects in the ChSz group in terms of their parental SES.

Overall, the present results indicate ChSz-specific NAA and Glx reductions in the mPFC in a single study that included subjects at 3 different stages of schizophrenia. These reductions are potentially related to changes in glutamatergic transmissions and regional neuronal integrity and may be related to the pathophysiology and progressive brain morphological changes seen in schizophrenia.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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